



UNIVERSITI PUTRA MALAYSIA

**CLONING OF GENES THAT ENCODE TRANSCRIPTION FACTORS
THAT BIND TO THE FLORAL CHITINASE GENE (CHI2;1) PROMOTER
OF TOMATO USING THE YEAST ONE-HYBRID SYSTEM**

CHAN PICK KUEN

FSMB 2003 29

**CLONING OF GENES THAT ENCODE TRANSCRIPTION FACTORS
THAT BIND TO THE FLORAL CHITINASE GENE (CHI2;1) PROMOTER
OF TOMATO USING THE YEAST ONE-HYBRID SYSTEM**

By

CHAN PICK KUEN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

August 2003



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

**CLONING OF GENES ENCODING TRANSCRIPTION FACTORS THAT
BIND TO THE FLORAL CHITINASE GENE (CHI2;1) PROMOTER OF
TOMATO USING THE YEAST ONE-HYBRID SYSTEM**

By

CHAN PICK KUEN

August 2003

Chairman : Associate Professor K. Harikrishna, Ph.D.

Faculty : Food Science and Biotechnology

Flowering plays an essential role in the plant's reproductive system and so has generated considerable research interest. Both the production and correct functioning of floral tissues are a prerequisite for the formation of seeds and fruits in all economically important agronomic and horticultural plants. Many flower-specific genes have been isolated and identified. Elucidation of the mechanisms that control flower-specific gene expression has led to the identification of their regulatory elements. These elements are useful for targeting other genes to floral organs at specific times during development. Targeting gene activity to specific floral tissues without affecting other portions of the flower is a very powerful tool for basic and applied studies. The ability to target specific gene expression is essential for discovery of function other related genes and for the selective manipulation of the flower. Some of the interesting characters for manipulation include reduced pollen allergenicity, increased flower longevity and flower numbers, and modified flower architecture. Previously, a promoter region of a tomato stylar endochitinase, Chi2;1 gene had been isolated (Harikrishna et al., 1996) and demonstrated to drive high level of expression in the pistil of transgenic plants. Hence, this study has been

tailored to isolate the transcription factors that bind to the Chi2;1 promoter and to identify specific binding regions within the promoter responsible for its binding.

The yeast one-hybrid system approach was used to isolate transcription factors that recognize elements within the Chi2;1 promoter. Out of 6.17×10^6 yeast transformants screened, thirteen putative positive clones were identified and isolated based on positive β -galactosidase assays. The DNA sequence of these clones was determined and compared to known DNA sequences in the GenBank database. Most of these clones did not have any significant homology with any known functional genes.

Expression studies were conducted on three clones, with two clones, LN2-1-1 and LN2-3-1 isolated from the -446 to -680 promoter region of Chi2;1 and one, 90-2-1 from the -211 to -445 region of the Chi2;1 promoter. LN2-1-1 and 90-2-1 are predominantly expressed in pistils at anthesis with lower expression in petals. Meanwhile, the expression of LN2-3-1 was detected in both vegetative and floral organs. The temporal and spatial expression patterns of LN2-1-1 and 90-2-1 were similar to Chi2;1 (Harikrishna et al., 1996). *In situ* hybridization of the LN2-1-1 and 90-2-1 clones revealed that the mRNA of both genes were localized to the transmitting tissue of the mature tomato pistil as with the mRNA of the Chi2;1 gene (Gasser et al., 1989). The binding ability of the proteins encoded by both of these genes to the respective DNA sequences has been shown through a mobility shift assay. Furthermore, the protein encoded by 90-2-1 has been shown to localize to the nucleus and bind specifically to a 20 base pair sequence within the Chi2;1 promoter. These results suggest that both LN2-1-1 and 90-2-1 might interact with the Chi2;1

promoter region and influence Chi2;1 gene expression to a certain extent. However, the functions of these genes in mediating style-specific expression is still to be confirmed by transgenic analysis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGKLONAN GEN-GEN YANG MENGEKODKAN FAKTOR-FAKTOR
TRANSKRIPSI YANG MENGIKAT PADA PROMOTER GEN BUNGA
ENDOKITINASE (CHI2;1) DARIPADA TOMATO MENGGUNAKAN
SISTEM YIS SATU-HIBRID**

Oleh

CHAN PICK KUEN

Ogos 2003

Pengerusi : Profesor Madya K. Harikrishna, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Bunga yang memainkan peranan penting dalam sistem reproduksi tumbuhan telah menjana minat yang mendalam terhadap penyelidikan. Penghasilan dan pengawalan fungsi bunga yang betul adalah wajib untuk penghasilan biji benih dan buah bagi semua tumbuhan yang penting dalam agronomi dan hortikultur. Banyak gen bunga-spesifik telah disaring and dikenalpasti. Penerangan mekanisma-mekanisma yang mengawal ekspresi gen bunga-spesifik telah membawa kepada penemuan elemen-elemen pengawalan. Elemen-elemen ini adalah penting untuk penyasaran gen-gen lain kepada organ bunga pada masa yang spesifik semasa tumbesaran. Sasaran aktiviti gen kepada organ-organ bunga yang spesifik adalah satu kaedah yang penting untuk penyelidikan asas dangunaan. Kebolehan untuk menyasar ekspresi gen yang spesifik adalah perlu bagi penemuan gen-gen yang mempunyai fungsi yang berkaitan dan manipulasi yang spesifik pada bunga. Antara ciri-ciri menarik untuk manipulasi termasuk pengurangan alergi terhadap debunga, penambahan jangka hayat bunga dan bilangan bunga dan pengubahsuaian arkitektur bunga. Sebelum ini, satu promoter gen endokitinase, Chi2;1 daripada pistil tomato telah

dipencilkan (Harikrishna et al., 1996) dan berupaya untuk mengarahkan ekspresi yang tinggi di tisu pemindahan bunga. Justru itu, kajian ini telah disesuaikan untuk pemencilan faktor-faktor transkripsi yang mengikat kepada promotor Chi2;1 dan mengenalpasti bahagian yang spesifik dalam promotor yang bertanggungjawab terhadap aktiviti-aktivitinya.

Sistem yis satu-hibrid (yeast one-hybrid system) digunakan untuk pemencilan factor-faktor transkripsi yang mengikat kepada promotor Chi2;1. Daripada jumlah transformasi yis sebanyak 6.17×10^6 yang disaring, tiga belas klon putatif positif telah dikenalpasti berdasarkan perubahan warna biru apabila diuji dengan kaedah asai ' β -galactosidase'. Jujukan klon-klon ini ditentukan dan perbandingan dengan bank data 'Genbank' telah dilakukan. Kebanyakan klon-klon ini tidak mempunyai persamaan dengan sebarang gen berfungsi.

Kajian ekspresi ke atas tiga klon dengan dua klon, LN2-1-1 dan LN2-3-1 yang dipencilkan daripada bahagian -446 ke -680 promotor Chi2;1 serta 90-2-1 daripada bahagian -211 ke -445 telah dilakukan. Klon LN2-1-1 dan 90-2-1 menunjukkan ekspresi yang tinggi pada tisu pistil yang matang dan ekspresi yang rendah pada petal bunga. Manakala ekspresi klon LN2-3-1 boleh dikesan pada tisu-tisu vegetatif dan bunga. Corak ekspresi klon LN2-1-1 dan 90-2-1 adalah serupa dengan Chi2;1 (Harikrishna et al., 1996). Hibridasi *in situ* klon LN2-1-1 dan 90-2-1 mengesahkan lokasi mRNA kedua-dua gen di tisu pemindahan bunga pada pistil tomato yang matang seperti mRNA Chi2;1 (Gasser et al., 1989). Keupayaan mengikat protein yang diekspresikan oleh kedua-dua gen ini ditunjukkan melalui asai 'mobility shift'. Selain daripada itu, protein yang diekspresikan oleh 90-2-1

adalah ditempatkan di nukleus dan mengikat spesifik kepada 20 jujukan DNA dalam promoter Chi2;1. Keputusan-keputusan yang diperolehi mencadangkan kedua-dua LN2-1-1 dan 90-2-1 berkemungkinan berinteraksi dengan promoter Chi2;1 serta mempengaruhi ekspresi Chi2;1 pada suatu tahap. Walaubagaimanapun, fungsi sebenar gen-gen ini di dalam ekspresi gen benangsari-spesifik perlu dikenalpasti melalui analisis transgenik.

ACKNOWLEDGEMENTS

First of all, I would like to express my heartiest gratitude and sincere appreciation to my supervisor, Assoc. Prof. Dr. K. Harikrishna for his guidance, advice and patience throughout this project. Special thanks are extended other members of my committee, Dr. Hirzun and Dr. Wong for their advice, comments and guidance whenever sought.

I would like to convey my special thanks to Dr. Ho for advice and guidance, Dr. Raha and Dr. Tan for letting me to use their equipment, Dr. Meilina and Dr. Sharifah (MPOB) for the *in situ* hybridization analysis, Dr. Vila (MARDI) for the use of the particle gun and Dr. Jenni for editing my project abstract for IAPTC&B fellowship.

I am thankful to members of the Genetic Lab; Mr Ong, Kak Dilla, Kak Liza, Choong, Siti Suhaila, Siti Habsah, See, Lee, Siaw San, Sew, Yang Ping, Mei Chooi, Yen Yen, Wai Har, Che Radziah, Tony, Yean Yee, Ken Jin and Teo for their assistance and guidance. Thanks also to Yiap, Chyan Leong, Varma, Christina and Boon Keat for their help and support. To my friends, Zaidah, Geok Chiam, Yuen Tze, Raevathi, Lian Pey, Poh Geok and Guan, thank you for your constant support and companionship.

Lastly, I wish to express my deepest gratitude and appreciation to my family especially my parents for their constant support throughout my studies.



I certify that an Examination Committee on 13th August 2003 to conduct the final examination of Chan Pick Kuen on her Doctor of Philosophy thesis entitled "Cloning Of Genes That Encode Transcription Factors That Bind To The Floral Chitinase Gene (Chi2;1) Promoter Of Tomato Using The Yeast One-Hybrid System" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the examination Committee are as follows:

Norihan Mohd Saleh, Ph.D.

Associate Professor,
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia
(Chairperson)

Harikrishna Kulaveerasingam, Ph.D.

Associate Professor,
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia
(Member)

Hirzun bin Mohd Yusof @ Hassan, Ph.D.

Lecturer,
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia
(Member)

Clemente Michael Wong Vui Ling, Ph.D.

Lecturer,
Department of Biotechnology,
Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia
(Member)

Peter M. Gresshoff, Ph.D.

Professor,
Department of Botany,
The University of Queensland,
(External Examiner)



GULAM RUSUL RAHMAT ALI, Ph.D.

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 29 OCT 2003

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Harikrishna Kulaveerasingam, Ph.D.

Associate Professor
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairman)

Hirzun bin Mohd Yusof @ Hassan, Ph.D.

Lecturer
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Clemente Michael Wong Vui Ling, Ph.D.

Lecturer
Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)



AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **14** NOV 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not previously or concurrently submitted for any other degree at UPM or other institutions.

Chan Pick Kuen

(CHAN PICK KUEN)

Date: 9/11/2003

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
 CHAPTER	
 1 INTRODUCTION	 1
 2 LITERATURE REVIEW	 4
2.1 Tomato	4
2.2 The Flower Structure of Tomato	5
2.3 Plant Chitinase	6
2.4 Regulatory Elements in Eukaryotic Genes	9
2.4.1 Promoter	9
2.4.2 Enhancer	11
2.4.3 Silencer	11
2.4.4 Insulator	11
2.4.5 Response Elements	11
2.4.6 <i>Trans</i> -acting Factors	12
2.5 Regulation of Gene Expression	13
2.6 Plant Transcription Factors	17
2.6.1 Classification of Plant Transcription Factors	18
2.6.2 Oligomerization Domains	21
2.6.3 Transcriptional Regulatory Domains	21
2.6.4 Nuclear Localization Signals (NLS)	22
2.7 Transcriptional Activation Domain	23
2.8 Chromatin Remodeling	27
2.9 Protein-DNA Interactions	28
2.9.1 Equilibria and Thermodynamic of Protein-DNA Interactions	31
2.9.2 Structural Analysis on the Protein-DNA Interactions	33
2.10 Suggested Model For Floral Regulation	39
2.11 Yeast One-hybrid	40
 3 MATERIALS AND METHODS	 42
3.1 Materials	42
3.2 Construction of Reporter Plasmids for Yeast One-Hybrid Screening	42
3.2.1 Preparation of Reporter Plasmids	42



3.2.2	Integration of Reporter Plasmid Constructs Into Yeast Genome	44
3.2.3	Small-Scale Yeast Transformation	45
3.2.4	Testing New Reporter Strains for Background Expression	46
3.2.5	β -Galactosidase Filter Assay	47
3.2.6	PCR Amplification	47
3.3	Construction of Tomato Pistil Activation Domain-Tagged cDNA Library	48
3.3.1	Extraction of Total RNA	48
3.3.2	mRNA Isolation using μ MACS mRNA Isolation Kit	49
3.3.3	Synthesis of Double-stranded cDNA	50
3.3.4	Blunt Ending the cDNA Termini	50
3.3.5	Ligation of <i>EcoR</i> I Adaptor	50
3.3.6	Phosphorylation of <i>EcoR</i> I Ends	51
3.3.7	Size Fractionation	51
3.3.8	Packaging	51
3.3.9	Titering the Primary Library	52
3.3.10	Library Amplification	53
3.3.11	<i>In vivo</i> Excision	53
3.3.12	Amplification of the Excised Phagemid Library	55
3.4	Library-Scale Yeast Transformation	55
3.5	Recovery of Plasmid Construct from Yeast	56
3.6	Preparation of Electro-Competent Bacteria Cells	57
3.7	Rapid Preparation of Plasmids by Boiling Lysis	58
3.8	Reverse Northern	58
3.8.1	Southern Blot	58
3.8.2	Synthesis of Double Stranded cDNA	60
3.8.3	Reverse Northern Hybridization	61
3.9	Yeast Protein Extraction	62
3.10	Protein Expression and Purification	62
3.11	Polyacrylamide Gel Electrophoresis	63
3.12	Coomassie Blue Staining of Polyacrylamide Gel	64
3.13	Electrophoretic Mobility Shift Assays	65
3.14	SDS Detection Method for Biotinylated Probes	65
3.15	Northern Hybridization	66
3.16	Extraction of Genomic DNA	67
3.17	Southern Hybridization	68
3.18	Intracellular localization of Proteins	68
3.19	<i>In Situ</i> RNA Hybridization	70
3.19.1	Sample Preparation	70
3.19.2	Probe Preparation	72
3.19.3	Prehybridization and Hybridization	73
3.19.4	Post-Hybridization Steps	74
3.19.5	Immunological Detection	75
3.19.6	Microscopy	75
4	RESULTS AND DISCUSSION	76

4.1	Construction of Reporter Plasmids for Yeast One-Hybrid Screening	76
4.2	Construction of Tomato Pistil Activation Domain-Tagged cDNA Library	80
4.3	Yeast One-Hybrid Screening of Tomato Pistil cDNA Library	82
4.4	Sequence Analysis of the Putative Clones	87
4.5	Expression Pattern of Putative Clones	98
4.6	Electrophoretic Mobility Shift Assays (EMSA) of Putative Clones	104
4.7	Localization of LN2-1-1 and 90-2-1 in Plant Cell	108
4.8	Localization of Gene Expression to Specific Tissues	110
5	CONCLUSION	115
	BIBLIOGRAPHY	118
	APPENDICES	132
	Appendix A: Circular Maps and Polylinker Sequence of Vectors	132
	Appendix B: Formulation for Media and Solutions	138
	Appendix C: MS Media	141
	Appendix D: Calculation for Probe Hydrolysis	142
	VITA	143

LIST OF TABLES

Table		Page
1	Size distribution of Chi2;1 promoter fragment.	44
2	Screening and characterization of cDNA library.	87
3	Summary of the sequence analysis of the putative clones.	90

LIST OF FIGURES

Figure		Page
1	Sequence of Chi2;1 promoter. Underlined is the TATA box	43
2	Tomato flower structure at anthesis.....	77
3	Tissue specific expression of the Chi2;1 gene in the style of tomato pistil.....	78
4	Integrative transformation of pLacZi into yeast genome introduced URA3 as a selectable marker at the chromosomal site.....	81
5	Estimating the percentage of recombinant clones by insert-screening.....	83
6	Colorimetric assay for β -galactosidase activity.....	84
7	Restriction profiling of the cDNA inserts of the thirteen clones.....	88
8	Nucleotide and deduce amino acid sequences of clone LN2-1-1.....	91
9	Hydropathy plot (Kyte-Doolittle, 1982) of LN2-1-1 with a calculated pI of 9.25.....	92
10	Nucleotide and deduce amino acid sequences of clone LN2-3-1.....	94
11	Hydropathy plot (Kyte-Doolittle, 1982) of LN2-3-1 with a calculated pI of 5.23.....	95
12	Nucleotide and deduce amino acid sequences of clone 90-2-1.....	96
13	Hydropathy plot (Kyte-Doolittle, 1982) of 90-2-1 with a calculated pI of 5.44.....	97
14	Alignment of 90-2-1 with three plant PHD proteins and one of human PHD protein.....	97
15	Northern analysis of LN2-1-1, LN2-3-1 and 90-2-1.....	100
16	DNA gel blots analysis of genomic sequences.....	102
17	EMSA of LN 2-1-1 and LN2-3-1 with Fragment 2 of Chi2;1 promoter.....	105
18	Identification of a distinct region within the Fragment 5 promoter that binds 90-2-1 protein	107



19	Cellular localization of LN2-1-1 and 90-2-1	109
20	<i>In situ</i> hybridization analysis of pistil longitudinal-sections	111
21	<i>In situ</i> hybridization analysis of mature tomato pistil cross-sections	112



LIST OF ABBREVIATIONS

<u>Symbol</u>	<u>Description</u>
α	alpha
β	beta
λ	lambda
%	percentage
$^{\circ}\text{C}$	degree Centigrade
<i>g</i>	gravity
AD	activation domain
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	base pair
BSA	bovine serum albumin
Ci	curie
C-terminal	carboxyl terminal
Cys	cysteine
cDNA	complementary DNA
DAPI	4',6-diamidino-2-phenylindole
DIG	Digoxigenin
DNA	deoxyribonucleic acid
DNase I	deoxyribonuclease
dNTP	deoxynucleotides
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'-deoxy-cytidine-5'-triphosphate
dGTP	2'-deoxy-guanosine-5'-triphosphate



dTTP	thymidine-5'-triphosphate
DEPC	diethyl pyrocarbonate
DMF	N,N-dimethylformamide
DMSO	dimethylsulphonyl oxide
DTT	dithiothreitol
EtBr	ethidium bromide
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol bis-(β -aminoethyle ether)
g	gram
GUS	β -glucuronidase gene
HCl	hydrochloric acid
His	histidine
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
hr	hours
IPTG	isopropyl- β -D-thiogalactoside
LB	Luria-Bertani
Leu	leucine
k	kilo
kb	kilobase-pair
KCl	potassium chloride
kD	kilodalton
L	liter
Leu	leucine
LiAc	lithium acetate
LiCl	lithium chloride

M	molar
mA	milliampere
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
MS	Murashige and Skoog medium
MgCl ₂	magnesium chloride
MgSO ₄	magnesium sulphate
MOPS	3-(N-morpholino) propane-sulphonic acid
mRNA	messenger RNA
NaCl	sodium chloride
NaOH	sodium hydroxide
NBT	nitro blue tetrazolium
ng	nanogram
N-terminal	amino terminal
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffer saline
PCR	polymerase chain reaction
pfu	plaque forming unit
pI	isoelectric point
PEG	polyethylene glycol

PMSF	phenylmethyl-sulfonyl fluoride
poly A ⁺ RNA	polyadenylated RNA
psi	pounds per square inch
PVP	polyvinylpyrrolidone
PVPP	polypolyvinylpyrrolidone
RNA	ribonucleic acid
rRNA	ribosomal RNA
RNase	ribonuclease
rpm	revolutions per minute
RT	reverse transcriptase
sec	second
SD	synthetic dropout
SDS	sodium dodecyl sulfate
Streptavidin-AP	Streptavidin-Alkaline Phosphatase
TAE	tris acetate EDTA
TBE	tris borate EDTA
TE	tris-EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
U	unit
μl	microliter
μg	microgram
μm	micrometer
Ura	uracil
UV	ultra violet

V	volt
X-Gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside
X-Gluc	5-bromo-4-chloro-3-indolyl-glucuronide

CHAPTER 1

INTRODUCTION

One of the many challenges in molecular genetics is to understand the molecular basis of gene regulation, as it involves the complex interaction of several components. The ability to regulate the expression of genes is fundamental to most biological phenomena such as development, differentiation, cell growth and responses to environmental signals. Transcriptional regulation of gene expression relies on the recognition of a *cis*-acting element by a transcription factor of the corresponding gene. The correct modulation of gene expression results in normal growth and development of an organism. Therefore, there has been a growing interest in gene regulation especially with tissue-specific genes. In tissue-specific gene regulation, a sequence specific DNA binding protein recognizes a *cis*-acting element of the corresponding gene. This binding will facilitate other components of the transcriptional machinery to initiate mRNA synthesis (Weinzierl, 1999).

Flowers play an essential role in the life cycle of flowering plants. Flowering is the first introductory step to fruit formation and is a fundamental part of the plants reproductive system. The range of events involved in flower development make flowering an excellent model system for understanding plant development in general. Understanding the nature of the protein products of tissue-specific genes and regulatory factors that control their temporal and tissue-specific expression will provide important insights into the mechanisms of flower formation and function.

The isolation and identification of regulatory regions of a floral tissue-specific gene, in conjunction with plant transformation will allow the targeted expression of genes to floral organs at specific times in their development. Introduction of these chimeric genes (appropriate promoter and desired gene) into plants will lead to better specific production of the gene product in floral organs.

The promoter concerned with in this study regulates a tomato floral stylar endochitinase, Chi2;1 (Harikrishna et al., 1996). The endochitinase was found to be expressed along the transmitting tract of the tomato style. Although the function of Chi2;1 has not been established, it is hypothesized to be involved in either facilitating pollen tube growth in the transmitting tissue or as a protective compound against pathogen (fungal) attack.

The Chi2;1 promoter was demonstrated to drive high level expression in the pistils of transgenic plants. The SK2 promoter, a potato homologue of the tomato Chi2;1 promoter, has been characterized by Ficker et al., in 1997. They have found that the regulatory elements directing expression were located within a 230 bp fragment. Therefore, it is anticipated that the promoter sequences of these two endochitinase genes would have high homology. The regulatory elements of the Chi2;1 promoter that interact to confer tissue-specific expression could possibly be located around the same region as the SK2 promoter.

It is hoped that the transcription factors that bind to specific *cis* regulatory elements can be identified and attempts to regulate genes through chimeric promoters in plants may be feasible through the use of bioinformatics and molecular